

of the inserts in selected clones was determined by restriction digest analysis and confirmed by sequencing (Sequenase 2.0, USB). This procedure resulted in the isolation of the expression plasmids used, pPKR-AS (containing the PKR cDNA in an antisense orientation under the control of the CMV promoter in the vector) and p[Arg²⁹⁶]PKR (containing the Arg²⁹⁶PKR cDNA under the control of the CMV promoter in the vector).

Example 2: Isolation of PKR-deficient stable transfectants

[0047] Stable transfectants were obtained by electroporation of 5×10^6 exponentially growing U937 cells with 10 μ g of each plasmid, in serum-free RPMI-1640 containing DEAE-dextran (50 μ g/mL), with a Gene Pulser apparatus (BioRad) set at 500 μ F, 250V. Bulk populations of stable transfectants were obtained by selection with 400 μ g/mL geneticin (GIBCO-BRL) for 3 weeks. Clonal lines were subsequently obtained by limiting dilution cloning. Cell lines were cultured in RPMI-1640 containing 10% fetal calf serum (complete media) and geneticin.

[0048] Five representative cell lines were selected for initial characterization: "U937-neo" (also called U9K-C) was the control cell line transfected with the parental vector, pRC-CMV; "U937-AS1" (also called U9K-A1) and "U937-AS3" (also called U9K-A3) were independent clones transfected with pPKR-AS; "U937-M13" (also called U9K-M13) and "U937-M22" (also called U9K-M22) were independent clones transfected with p[Arg²⁹⁶]PKR.

Example 3: Characterization of PKR-deficient transfectants

[0049] PKR kinase activity was measured in an autophosphorylation assay that uses poly(I):poly(C)-cellulose for binding and activation of PKR enzyme. PKR autophosphorylation assay was performed essentially as described by Maran et al. with the following modifications. Cell extracts (100 μ g of protein per assay) were incubated with poly(I):poly(C)-cellulose for 1 hour on ice, washed three times, and incubated for 30 minutes at 30°C in 50 μ l of a reaction buffer (20 mM HEPES (pH 7.5), 50 mM KCl, 5 mM 2-mercaptoethanol, 1.5 mM Magnesium acetate, 1.5 mM MnCl₂) containing 1 μ Ci of [γ -³²P]ATP. Proteins were separated on a 10% SDS- polyacrylamide gel and analyzed by autoradiography.

[0050] Cell extracts from IFN-treated HeLa and mouse L929 cells were used as positive controls, since PKR activity in these cells has been previously characterized (Meurs et al.) (FIG. 1A, lanes 1 and 8). U937-neo cells contained low basal levels of PKR activity which